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ANTIMICROBIAL STUDIES OF CRUDE EXTRACTS OF SELECTED MEDICINAL PLANTS

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Abstract

The antimicrobial activity of crude extracts of Ocimum sanctum (OS), Ocimum kilimandsacharicum (OK), Garcinia mangostana (GM), and Swertia chirayata (SC) was evaluated on selected enteric pathogens. The antibacterial and antifungal activity of the extracts at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The tested plant extracts showed promising antimicrobial activity, thus justifying their traditional use. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested.

Keywords Antibacterial, Antifungal, Medicinal plants

1. Introduction

Medicinal plants are widely used for curing various diseases since traditional times. Different plant parts like root, leaves, stems, seeds or even whole plants are known to have therapeutic potentials [1]. Medicinal plants have been used as preservatives, in pharmaceutics, natural therapies etc. There is a greater demand for medicinally important plants &cultivation of such plants is recommended. About 100 plant species are involved in 25% of all prescribed drugs in advanced countries [2].Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [3]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts which can be used are root, stem, flower, fruit, and twigs and modified plant organs. Although many plant species have been tested for antimicrobial properties, the majority of them have not been adequately evaluated and tested for various diseases [4].

Diarrhoeal infections are found to be major health problems in most of the developing countries. Enteric bacteria comprised of E. coli, Pseudomonas sp., and S. aureus, Salmonella sp., Shigella sp., Proteus sp.,etc. which are major diagnostic agents of occasional and epidemic diarrhoea both in small children and in adults[5].Resistance to antibacterial agents has important implications for mortality and health care in the community. So it makes it necessary to discover new classes of compounds to treat infections. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Plants are rich

source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, alkaloids, and flavonoids, which are reported to have in vitro antimicrobial properties. [6, 7]

Fatal infectious diseases are the world's leading cause of concern with drug & antibiotic resistant human pathogenic bacteria commonly reported from all over the world [8-12]. Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [13]. There is a constant need for new and effective therapeutic agents. There is a need to develop alternative newly synthesized antimicrobial substances or substance from other sources including plants[14].With increasing use of drugs, microorganisms are attaining resistance to commonly used antibiotics, which leads to downfall of effectiveness of conventional medicines and therefore, search for new antimicrobial agents has become necessary[15]. Plants having antimicrobial activity have attracted attention in recent years [16-18].

A pathogenic microorganism causes infectious diseases due to presence of microorganisms like, viruses, fungi, bacteria and multicellular parasites. These diseases can be transmitted from one individual to another and are called as communicable or transmissible diseases[19] .

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, an evaluation to screen the local flora for antibacterial and antifungal activity of methanolic and aqueous extracts of Ocimum sanctum(OS), Ocimum kilimandsacharicum(OK),Petroleum ether, dichloromethane and chloroform extract of Garcinia mangostana (GM),and Petroleum ether extract of Swertia chirayata (SC) was undertaken to find the zone of inhibition.

2. Methodology

Test organisms taken for study were Staphylococcus auras, Bacillus subtilis, Escherichia coli, Aspergilli awamorii, Rhizopus species and Candida albicans.

Determination of zone of inhibition

In vitro antibacterial activity was determined by using Mueller Hinton Agar while in vitro antifungal activity was determined by using Sabouraud Dextrose Agar obtained from Himedia Ltd., Mumbai. Twenty-four hours old culture of selected bacteria/fungi was mixed with physiological saline and the turbidity was corrected by adding sterile physiological saline and subcultured on Sabouraud Dextrose and suspended in sterile distilled water to an absorbance of 0.6 at 450 nm. Freshly prepared suspensions in sterile water (Optical Density: 0.6) of pure isolated cultures of Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Aspergillis awamorii, Rhizopus species and Candida albicans were mixed with the sterilized Hinton agar and Sabouraud dextrose agar maintained at 42.0±2.0 ^{0}C.Petri plates were prepared by pouring 10 ml of Mueller Hinton Agar for bacteria and Sabouraud Dextrose Agar for fungi containing microbial culture was allowed to solidify. Five wells of 6 mm diameter were bored in the medium with the help of sterile cork-borer having 6 mm diameter and were labelled properly and 100, 200/250 and 400/500 µg/mL of the working solution / vehicle and same volume of extraction solvent for control, as well as 25 µg/mL

of the standard (Bacitracin /Ciprofloxacin) was filled in these wells with the help of micropipette. Similar sets were made for other extracts. The discs were then applied and the plates were incubated at 37±2.0 ^{0}C for 2 days and 24±2.0 ^{0}C for 5 days respectively. Plates were observed for zone of inhibition zone was measured as diameter in four directions and expressed as mean.

3. Results and Discussion

The antibacterial activity of the extracts at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter.

Diameter of the zone of inhibition (ZOI) was measured for the estimation of potency of the antimicrobial substance which is indicated in Table 1.

Table 1: Zone of inhibition (in mm)

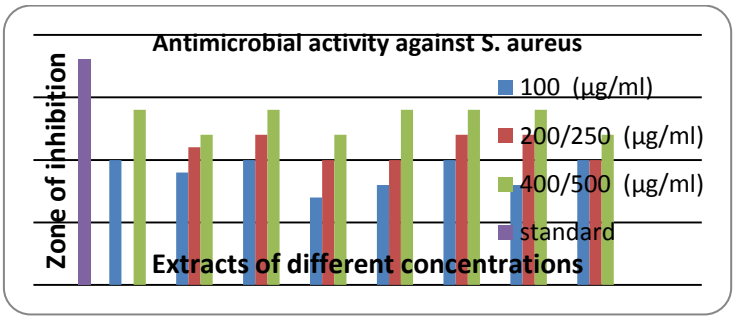
S. No	Extract	Solvent	Concentration µg/mL	S. aureus	B. subtilis	E. coli	Aspergillus awamorii	Rhizopus sp	C. albicans
1	Standard		25	18	16	20			16
2	M1	Petroleum ether	100 250 500	10 - 14	-	8 11 13	8 11 12	7 9 10	-
3	M2	dichloromethane	100 250 500	9 11 12	-	7 10 12	12 13	8 10 12	-
4	M3	chloroform	100 250 500	10 12 14	-	9 11 14	7 10 12	-10 11	-
5	S1	Petroleum ether	100 250 500	7 10 12	-	10 12 13	- 9 11	10 12 14	-
6	O1	Methanol	100 200 400	8 10 14	- 8 8	10 12 10	-	-	10 12 14
7	K1	Methanol	100 200 400	10 12 14	- 8 10	12 16 16	-	-	10 10 16
8	O2	Water	100 200 400	8 12 14	- 8 10	12 12 14	-	-	8 10 10
9	K2	Water	100 200 400	10 10 12	- - 10	10 10 14	-	-	10 12 16

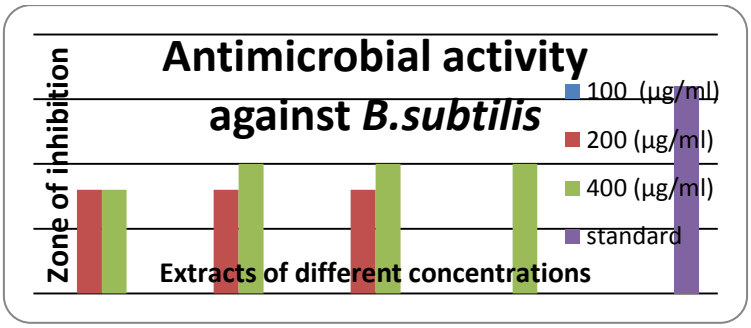
Where, M1, M2, M3 – Petroleum ether, dichloromethane and chloroform extract of G. mangostana, S1 – Petroleum ether extract of S. chirayita, O1, O2 –methanolic and aqueous extract of O. sanctum K1, K2-methanolic and aqueous extract of O.kilimandsacharicum

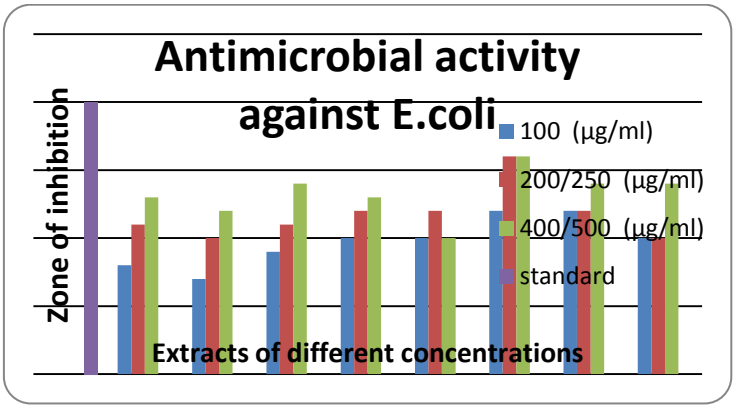
From Table 1, it can be seen that the extract M1 and M3 showed strong antimicrobial activity against S. aureus than M2 and M3 at 500 µg/ml. In case of activity of extract against E.coli, all the extracts were active at 500µg/ml. Extracts M1 and M3 have shown good inhibition zone against Aspergillus awamorii. Fungal strain of Rhizopus species have shown good inhibition zone by S1 as

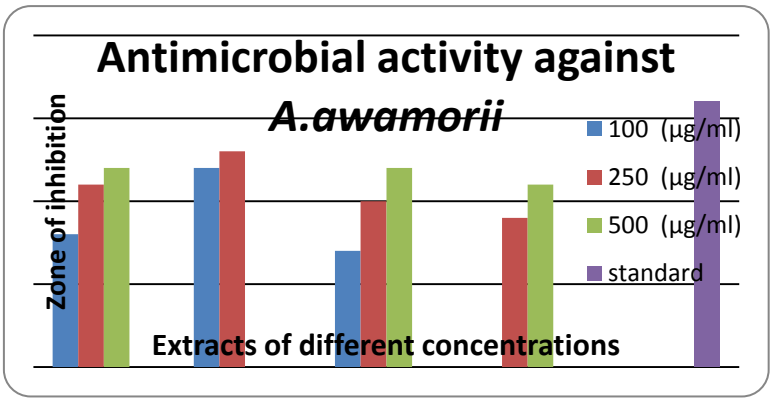
compared to other extracts. Extract O1 showed strong antimicrobial activity against S.aureus and C. albicans at 400 µg/mL concentrations and moderate activity against E. coli and B. subtilis. Extract K1showed strong antimicrobial activity against S. aureus, E. coli and C. albicans at higher concentration. Extract K1showed moderate activity against B. subtilis. Extracts O2 showed strong antimicrobial activity against S.aureus and moderate against B.subtilis, E. coli and C. albicans. Whereas Extract K2 showed moderate activity against E. coli, S.aureus & B.subtilis only at high concentration, and strong activity against C. albicans at higher concentration. Zone of inhibition of various extract against microorganism are represented in graphs.

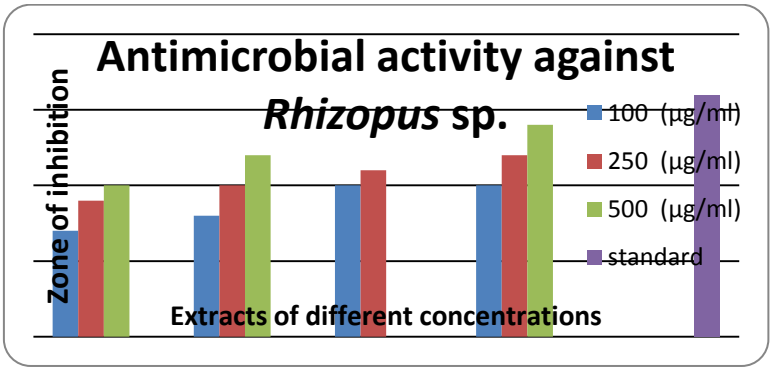
Graphs

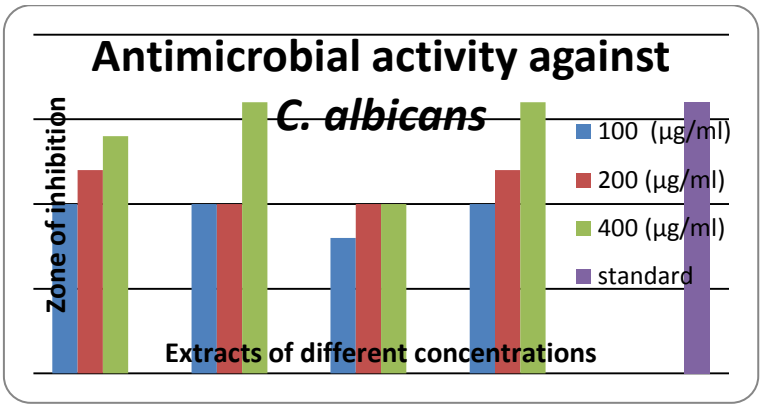












4. Conclusion

In conclusion, the results of zone of inhibition of extracts observed in different plant parts suggest that they show strong antimicrobial activity against S. aureus, E. coli as compared to other microorganisms. The activity against microorganisms of plant extracts of Ocimum sanctum (OS), Ocimum kilimandsacharicum (OK), Garcinia mangostana (GM), and Swertia chirayata (SC) can be used for curing enteric and diarrhoeal diseases. Medicinal plants used in treating enteric diseases were found to be very useful as compared to synthetic antibiotics against bacterial infections. Many medicinal plants and their extracts are found to be alternate and safe source of chemical constituents for preparing formulations and pharmaceutical products without side effects. It is clear from the zone of inhibition of extracts that they are effective for treatment of bacterial diseases and can be used as antimicrobial agents, but further work is necessary to find the minimum inhibition

concentration as well as clinical safety of extracts so as to make them safe, authentic and cost effective in treating various bacterial diseases.

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